Bacteria in the lakes of the Tibetan Plateau and polar regions

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Abstract

The Tibetan Plateau, also termed 'the Third Pole' harbors the largest number of high-altitude lakes in the world. Due to the presence of extreme conditions such as low temperature and oligotrophy, the lakes of the Tibetan Plateau share environmental features in common with lakes in the polar regions. However, the extent to which these environments are analogous, or indeed whether they harbor similar microbial communities or a high level of endemic species is poorly understood. Here we compared high-throughput 16S rRNA gene sequencing data from the lakes of the three different regions in order to characterize their taxonomic diversity, the community composition and biogeography. Our results showed despite the similarity in environmental conditions, the spatial distribution of the bacterial communities was distinct with only 3.1% of all operational taxonomic units (OTUs) being present in all three regions (although these OTUs did account for a considerable proportion of the total sequences, 36.4%). Sequences belonging to Burkholderiales and Actinomycetales dominated the shared OTUs across all three regions. Scale dependent distance decay patterns provided evidence of dispersal limitation. Climatic variables and dispersal limitation were apparently both important in controlling the spatial distribution of bacterial communities across regions. This work expands our understanding of the diversity and biogeography of lake bacterial communities across the Tibetan Plateau and provides insights into how they compare to those of the Antarctic and Arctic.

Keywords: tThe Tthird Ppole; lLake bacterial communities; sSpatial distribution; dDispersal limitation

1.1 Introduction

The Tibetan Plateau, also termed 'the Third Pole' (Qiu, 2008), harbors the largest number of high-altitude lakes (average elevation of more than 4000 m above sea level) in the world (Zhang et al., 2011). There are about 32,843 lakes with a total area of 43,151.08 ± 411.49 km^2 on the Tibetan Plateau, and these lakes span a diverse range of environmental conditions, from dilute, glacier-fed meltwaters to hypersaline lakes and seasonal or perennially ice-capped, stratified lakes (Zhang et al., 2014). The Tibetan lakes, especially those glacier-fed lakes, that have the characteristic of appearing/disappearing and expansion/shrinkage, have been suggested to be sensitive indicators of climate change (Zhang et al., 2017).

Lakes in the Tibetan Plateau are commonly characterized by low temperature and limited nutrient availability, but being remote from human disturbance, which is similar to what has been reported for the Antarctic and Arctic lakes (Liu et al., 2014; Morgan-Kiss and Li, 2019; Schütte et al., 2016). These common features make lakes in the three regions a unique natural laboratory to study the microbial distribution patterns at both regional and global scales. Previous studies have revealed that bacterial communities in the Tibetan lakes are dominated by Proteobacteria and Actinobacteria, and are primarily regulated by salinity-driven environmental filtering and geographic distance (Liu et al., 2019a, 2019b; Liu et al., 2020; Yang et al., 2016). It is well established that the local extreme habitat conditions including near freezing temperatures and oligotrophy, are key controlling factors in the development and assembly of bacterial communities in the Antarctic lakes (Kwon et al., 2017; Morgan-Kiss and Li, 2019). In the Arctic regions, bacterial communities have been reported to correlate with nitrogen and phosphorus availability, dissolved organic carbon, dissolved oxygen and conductivity in lakes (Haukka et al., 2006; Somers et al., 2019). In addition, studies of bacterial communities from both the Antarctic and Arctic lakes have revealed that geographical distance and local limnological profiles (e.g., temperature, conductivity, pH and Chlorophyll) play an important role in shaping the spatial distributions of bacterial communities (Daniel et al., 2016). However, up to now no study has addressed whether the Tibetan lakes, that share some features with lakes from Arctic and Antarctica, share any similarities with polar lakes in terms of their bacterial communities.

Regional-scale comparative studies on lake bacterial communities are important in providing the understanding of basic questions of aquatic microbial ecology, such as how communities vary across spatial scales and environmental gradients. Several recent studies have shown a very important effect of local environmental conditions such as pH (Ortiz-Álvarez et al., 2020) and salinity (Logares et al., 2013), and hydrographic factors such as water residence time and connectivity (Niño-García et al., 2016; Ruiz-González et al., 2015) in explaining spatial variations in lake bacterial communities at the regional scale. Despite the substantial efforts that have been made in disentangling the mechanisms shaping lake bacterial communities within relatively homogeneous regions, there have been few studies focus on the possible diverse response of lake bacteria to climate change between regions that have different regional climates and geomorphological conditions (Daniel et al., 2016).

Evidence of the effects of climate change is clear across the Tibetan Plateau, Antarctic and the Arctic regions (Bockheim et al., 2013; Lehnherr et al., 2018; Yao et al., 2019), as increases in air temperature lead to enhanced glacial melt and permafrost thaw across all three regions. Lake systems in the three regions are experiencing shifts in ice-cover duration, temperature, light and nutrient availability and geochemical properties, which are expected to have profound effects on their bacterial communities. Microorganisms are already known to display biogeographic patterns in various aquatic ecosystems, such as mountain streams (Wang et al., 2012), rivers (Isabwe et al., 2019; Wang et al., 2015), lakes (Logares et al., 2018; Soininen et al., 2011) and seawater (Liu et al., 2019a, 2019b; Wu et al., 2018). Previous studies have reported that species sorting, primarily driven by climatic variables, and dispersal limitation are the two major processes that determine the biogeography of microbial communities at the regional or global scale (Hanson et al., 2012 ; Langenheder and Lindstrom, 2019; Yang et al., 2019). It also has been proposed that continuing climate change may weaken biogeographic boundaries, which further weakens spatial effects in the polar regions (Kleinteich et al., 2017). However, we have yet to understand the relative contributions of climatic and spatial variables in structuring lake microbial communities across the Tibetan Plateau, Antarctic and the Arctic regions.

In this study, we collected 84 samples from 19 Tibetan lakes combined with public data obtained from NCBI Sequence Read Archive database to present the results of a meta-analysis of sequencing-based studies on the diversity and biogeography of bacterial communities in the Tibetan Plateau, compared to Antarctic and Arctic lakes. Specifically, we aimed to answer the following questions: (i) Are there significant differences in the bacterial diversity and composition of the lakes of the Tibetan Plateau, when compared to Antarctic and Arctic lakes? (ii) Do the biogeographic patterns vary over regional and continental scales? (iii) to what extent might climatic variables and geographic distance explain these observations? We hypothesized that lake bacterial communities of the Tibetan plateau would show significant compositional differences to those of the Arctic and Antarctica given their geographic isolation and climatic conditions.

~~2.~~2 Materials and methods

~~2.1.~~2.1 Sampling and data collection

A total of 84 water samples used in this study were collected from 19 Tibetan lakes during several field campaigns in 2016 and 2017 summers, respectively (Dataset S1). The selected lakes covered an area from 75.05°E to 90.86°E longitudinally and 28.89°N to 38.46°N latitudinally. A minimum of triplicate surface water samples (~0.5 m depth) from the epilimnion were collected with a Schindler sampler at the center of each lake. For microbial DNA collection, 1 L water samples were filtered through a 20 μm mesh (Millipore, USA) to remove large particles or organisms, and subsequently through a 0.22 μm pore size polycarbonate filters (47 mm diameter; Millipore). The filters were immersed in 1.8 mL of RNAlater (Life Technologies, USA) to stabilize nucleic acids and then stored at −80 °C in the laboratory until further processing. Environmental DNA from each sample was extracted using the MP FastDNA~~TM~~™ SPIN DNA kit (MP Biomedicals, CA, United States) and amplified using primers targeting the V4 region of the 16S rRNA gene (515F (5′-GTGCCAGCMGCCGCGGTAA-3′, and 806R 5′-GGACTACHVGGGTWTCTAAT-3′) of the

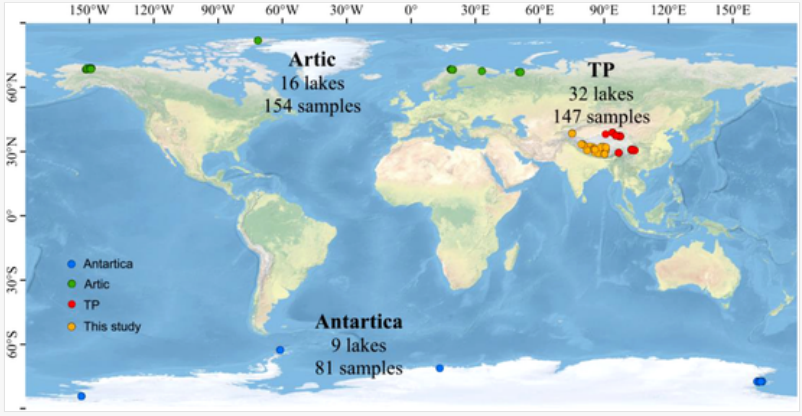
Earth Microbiome Project (EMP) (Caporaso et al., 2010). The amplicons were mixed in equimolar concentrations and sequenced on an Illumina MiSeq Sequencer (Illumina, San Diego, CA) with a paired-end strategy (2 × 250 bp). Web of Science (2010–2019) was searched for literature and the NCBI Sequence Read Archive database for deposited sequences using the combined keywords ('Antarctic' or 'Arctic' or 'Tibetan') and ('lake microbial' or 'lake bacterial'). Since cloning-based and high-throughput sequencing methods are not directly comparable, only studies or data generated from high-throughput sequencing methods were included. Based on the criteria described above, a total of 147 water samples were collected from 32 Tibetan lakes, 81 water samples from 9 Antarctic lakes and 154 water samples from 16 Arctic lakes (Fig. 1; Dataset S1). Due to poor accessibility and harsh field conditions, there were fewer studies or available datasets on Antarctic lakes than those in the Arctic and Tibetan Plateau. It should note that in the current study only planktonic communities were considered so that the three datasets are directly comparable.

~~2.2.~~2.2 Data processing

The "*fastq-dump*" function in the SRA Toolkit (v2.8.2) was used to download and extract fastq files from the NCBI Sequence Read Archive database according to the sample run ID. The paired-end fastq files were assembled with VSEARCH (v2.7.1) using the code "*vsearch fastq_mergepairs*" (Rognes et al., 2016). All of the selected high-throughput sequences were processed using the Quantitative Insights into Microbial Ecology pipeline (QIIME) (v1.9.0) (Caporaso et al., 2010). Briefly, the reads which had ambiguous bases and mismatches to the barcode or primers were discarded. After quality filtering, chimeric sequences were detected and removed using USEARCH (Edgar, 2010). The script "*pick_closed_reference_otus.py*" was used at the 99% sequence identity level against the SILVA 132 reference database (Quast et al., 2012) to generate an OTU table. Singleton OTUs (consisting of only one read) were removed from the downstream analysis. Taxonomic identity of each representative sequence was determined using the RDP Classifier (Wang et al., 2007). After taxonomies had been assigned, non-bacterial and chloroplast sequences were removed from the analysis. To enable comparisons between samples, the OTU table was randomly subsampled to ensure an equal number of sequences per sample, based on the sample with the lowest number of sequences (10,292 sequences).

alt-text: Fig. 1

~~Fig. 1.~~Fig. 1



Geographical distributions of 67 lakes at which water sample data were selected or collected from the TP (147 water samples from 32 lakes), Antarctica (81 water samples from 9 lakes) and the Arctic (154 water samples from 16 lakes), respectively. TP: Tibetan Plateau.

~~2.3.~~2.3 Climatic data extraction

Mean annual temperature (MAT, 2 m air temperature), mean annual precipitation (MAP), surface net solar radiation (NSR) and evaporation were extracted from ERA5 monthly averaged data on single levels from 2010 to 2019 according to the geographical locations of the collected lake samples. The extraction processes were conducted in the R environment (v3.6.1; http://www.r-project.org) using additional packages, such as "ncdf4", "raster", "rgdal" and "sp".

ERA5 is developed by the European Centre for Medium-Range Weather Forecasts (ECMWF) (https://www.ecmwf.int/) and freely available through the Copernicus Climate Data Store (https://cds.climate.copernicus.eu/). It is an atmospheric reanalysis model that provides a space and time resolution sufficient for meteorological studies ranging from the mesoscale to the global scale.

~~2.4.~~2.4 Statistical analyses

All extracted climatic variables, except the MAT, were log(x + 1) transformed for multivariate statistical analysis. We used the "*estimateR*" function in R package "vegan" to estimate the richness index of the bacterial communities (Oksanen et al., 2007). Multiple ordinary least squares regression was performed to examine the relationship between climatic variables and community richness pattern using SAM v4.0 (Rangel et al., 2010). Nonmetric multidimensional

scaling (NMDS) was used to visualize the overall differences in bacterial community composition based on Bray–Curtis distance (Clarke, 1993). We applied the "*envfit*" function in "vegan" package to fit climatic vectors onto the ordination space (Oksanen et al., 2007). Dissimilarity tests like multiple response permutation procedure (MRPP), analysis of similarities (ANOSIM) and permutational multivariate analysis of variance (perMANOVA) with Adonis function were then employed to evaluate the significance of the differences found in community composition between regions based on Bray–Curtis distance with 999 permutations (Anderson, 2001). Spearman's rank correlations were used to assess the relationships between bacterial community dissimilarity (Bray–Curtis distance) and geographic distance. Geographic distance was determined based on the geographic coordinates of sampling sites using function "*distGeo*" in R package "geosphere" (Hijmans et al., 2016). Mantel and partial Mantel tests were employed to assess the relationships between bacterial community composition (Bray–Curtis distance) and geographic distance or climatic variables (Euclidean distance) with 999 permutations in "vegan" package.

We performed additional analyses using 243 samples (81 samples from each region) based on the lowest number of samples from the Antarctic lakes to test whether the spatial pattern of bacterial communities were biased by sample numbers. Samples of the sub-dataset were chosen at random from duplicate samples of each lake within the Tibetan Plateau and Arctic regions.

~~3.~~3 Results

~~3.1.~~3.1 Spatial patterns of climatic variables

Multi-year average MAT, MAP, NSR and evaporation for the nine-year period (2010–2019) were significantly different between the Antarctic, Arctic and Tibetan Plateau (Kruskal-Wallis test, $P < 0.05$; Table S1 and Fig. S1). The MAT, MAP, NSR and evaporation were highest in the Tibetan Plateau (mean ± standard deviation; –0.47 ± 4.44 °C, 1781.58 ± 1064.25 mm, 16.20 ± 2.57 MJ m^{-2} and 1024.05 ± 442.45 mm, respectively) and lowest in the Antarctica (–23.23 ± 6.05 °C, 336.96 ± 610.06 mm, 3.32 ± 2.48 MJ m^{-2} and 86.36 ± 203.21 mm, respectively) (Table S1). The MAT, MAP, NSR and evaporation were significantly correlated with geographic location (latitude or longitude, Spearman's rank correlation, $P < 0.05$; Fig. S2).

~~3.2.~~3.2 Variation of bacterial OTU richness and community composition

After cleaning and quality-checked processing, a total of 44,750,779 qualified 16S rRNA reads were obtained from 382 water samples. These reads represented 134,371 OTUs at the cutoff of 99% sequence identity used in this study. The bacterial OTU richness of the Arctic lake samples was significantly higher than those of Tibetan Plateau and Antarctic lake samples (Kruskal-Wallis test, $P < 0.001$; Fig. 2). Considering the whole dataset, the taxonomic richness of the studied communities was significantly correlated with MAP as revealed by the multiple ordinary least squares regression analysis ($P < 0.01$; Table 1). For the Tibetan lake bacterial communities, MAP, MAT and evaporation were the variables correlated to community richness despite these variables provided relatively marginal explanatory power ($r^2 = 0.180$; Table 1). For the bacterial communities in the Antarctic lakes, MAP, MAT and NSR were the main variables explaining the variations in community richness ($r^2 = 0.386$, $P < 0.001$; Table 1), whereas richness of the Arctic lake bacterial communities was best explained by MAP, MAT and evaporation ($r^2 = 0.441$, $P < 0.001$; Table 1).

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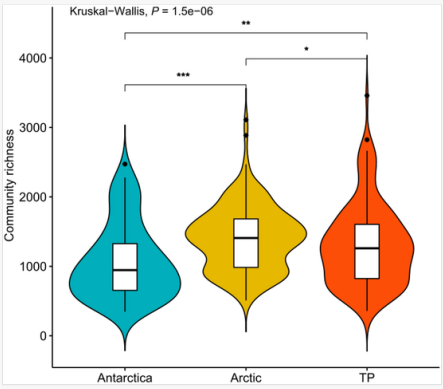
~~Fig. 2.~~Fig. 2



Comparison of the OTUs richness across the TP, Antarctic and the Arctic lakes. TP: Tibetan Plateau. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

alt-text: Table 1

~~Table 1.~~Table 1

Results of multiple ordinary least squares regression using Akaike's information criterion, correlating community richness with climatic variables. The spatial autocorrelation in the model residuals was considered. All of the variables were displayed with increasing *P*-values.

	Diversity index	r^2	*P*	n	Explanatory variables (beta weights)[a]
All	Richness	0.057	0.004	382	MAP (0.198)**
Antarctica	Richness	0.386	<0.001	81	MAP (−2.805)***, MAT (2.385)***, NSR (0.593)*
Arctic	Richness	0.441	<0.001	154	MAT (2.495)***, MAP (1.262)***, Evap (1.318)**
TP	Richness	0.180	<0.001	147	MAP (0.488)***, MAT (0.303)**, Evap (−0.233)*

n: number of samples.
All: the whole dataset, TP: Tibetan Plateau, NSR: surface net solar radiation, Evap: evaporation, MAT: mean annual temperature, MAP: mean annual precipitation.

Table Footnotes

[a] Standardized partial regression coefficients.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

NMDS analysis, based on the Bray–Curtis distance across lake samples, revealed that communities showed a certain structuring based on geographic location. (Fig. 3). Samples from the Tibetan Plateau and Arctic lakes showed a clear segregation, whereas the Antarctic lake samples were much more dispersed. The significance of distribution pattern was further confirmed by the dissimilarity tests (MRPP/ANOSIM/perMANOVA, all $P = 0.001$; Table S2). This spatial structuring of bacterial community composition was significantly influenced by MAT, MAP, NSR and evaporation (all $P = 0.001$; Fig. 3 and Table S3). NSR was the variable that best fitted the ordination space ($r^2 = 0.581$; Table S3).

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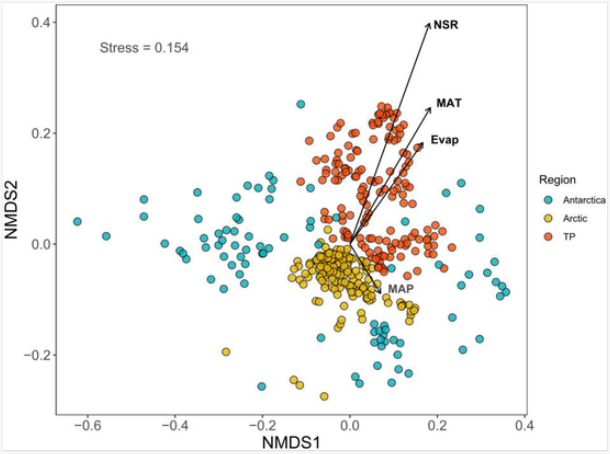
~~Fig. 3.~~Fig. 3



Nonmetric multidimensional scaling (NMDS) ordination of community dissimilarities across the TP, Antarctic and the Arctic lakes using Bray-Curtis distance. The arrows indicate the direction at which the climatic vectors fit the best (using “envfit” function) onto the NMDS ordination space. TP: Tibetan Plateau, MAT: mean annual temperature, MAP: mean annual precipitation, NSR: surface net solar radiation, Evap: evaporation.

The proportion of unique OTUs was highest in Arctic lakes (29.2%, 36,141 OTUs), followed by the Tibetan Plateau (26.4%, 32,742) and Antarctica (21.1%, 26,199 OTUs) (Fig. 4). In total, 3.1% (3826) of the OTUs with 36.4% (1,419,444 sequences) of total sequences were found in all three regions. 10.9% (13,551) of the OTUs were shared by the Tibetan Plateau and Arctic lake communities and 6.8% (8463) were shared by the Tibetan Plateau and Antarctic lakes.

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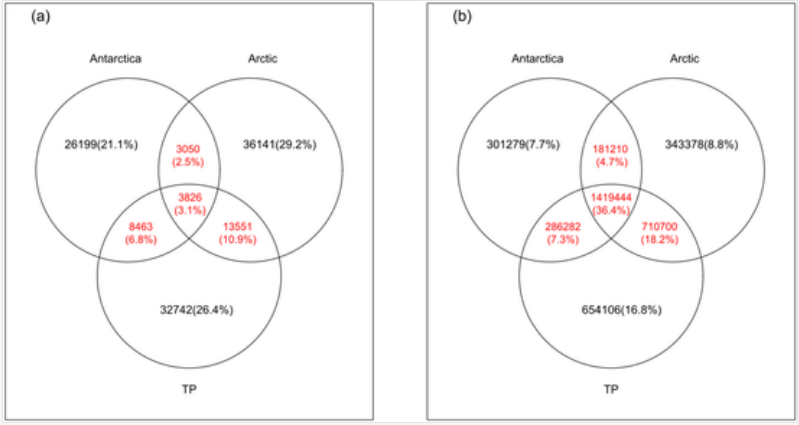
~~Fig. 4.~~Fig. 4



Venn diagram revealing showing (a) the number and proportion of shared and unique OTUs and (b) the number and proportion of shared and unique sequences across the TP, Antarctic and the Arctic lakes. TP: Tibetan Plateau.

Sequences belonging to Betaproteobacteria (33%), Actinobacteria (21%) and Bacteroidetes (14%) dominated the shared OTUs of the three regions (Fig. 5a). Sequences assigned to the phylum Bacteroidetes comprised the majority of taxa unique in the Antarctic (24%) and Tibetan Plateau (21%) lakes (Fig. 5b and ~~5~~d). Betaproteobacteria and Bacteroidetes dominated the unique OTUs in the Arctic lakes, accounting for 20% and 18% of all unique sequences, respectively (Fig. 5c). At the order level, Burkholderiales in the class Betaproteobacteria (29%) and Actinomycetales in the phylum Actinobacteria (20%) were the two most abundant bacterial assemblages in all three regions (Fig. S3a). However, Burkholderiales (16%) was primarily found in the Arctic lakes and Actinomycetales (15%) was primarily identified in the Tibetan lakes (Fig. S3c and 3d). Cytophagales (10%) and Flavobacteriales (9%) assigned to the Bacteroidetes phylum were the dominant orders in the Antarctic lakes (Fig. S3b). The orders Synechococcales and Nostocales affiliated with the phylum Cyanobacteria were present in high abundance in the Arctic lakes, while Oscillatoriales was present in a high abundance in the Antarctic lakes.

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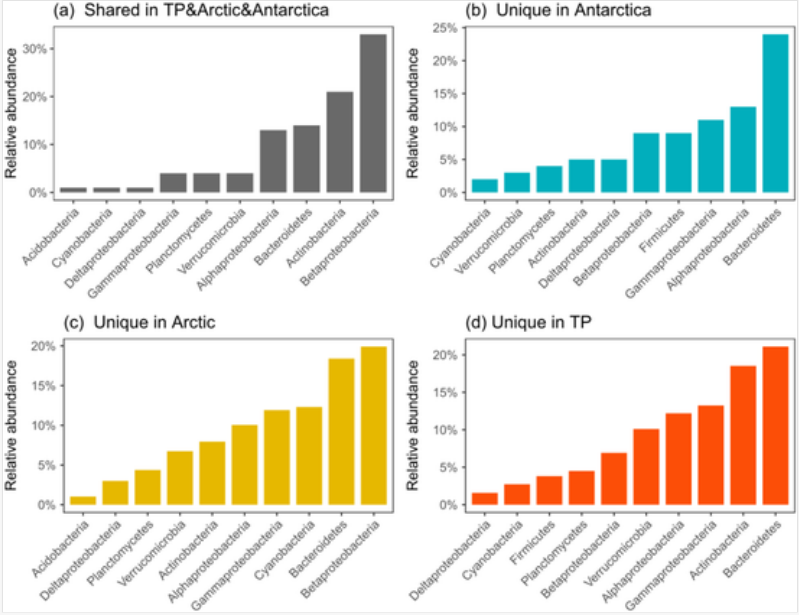

Distribution of abundant taxonomic groups in lakes of the Tibetan Plateau, Antarctica and the Arctic. (a) Top 10 abundant bacterial phyla/classes shared across the TP, Antarctic and the Arctic and lakes, (b-d) Top 10 abundant bacterial phyla/classes unique in the Antarctic, Arctic and the TP lakes, respectively. TP: Tibetan Plateau.

To avoid the potential bias caused by different samples collected from regions, 81 samples were chosen from each region based on the lowest number of samples from the Antarctic lakes and reanalyzed. Similar results were obtained when considering these 81 samples (Figs. S4, S5 and S6). The percentage of OTUs which were common to all three regions was smaller, while the relative fraction occurring uniquely within each region was larger (Fig. S4). The dominant bacterial groups of shared OTUs and unique OTUs in each region were similar to those of 382 samples at the phylum and order level (Figs. S5 and S6).

~~3.3.~~3.3 Spatial patterns of bacterial communities within and across regions

A significant negative relationship between geographic distance and community similarity was observed across all scales ($P < 0.001$; Fig. 6). The slope of the distance-decay relationship varied across different spatial scales. The slope of distance-decay curve within regions (slope =~~.~~0.054) was gentler than the overall slope (slope =~~.~~0.073), whereas the slope of distance-decay curve across regions was steeper than the overall slope (~~.~~0.450 versus ~~.~~0.076) (Fig. 6). We also observed a consistent decay of community similarity with geographic distance for bacterial communities within each region (Fig. S7). The slopes of distance-decay curve of the Tibetan lake bacterial communities (~~.~~0.074) was steeper than those of Antarctic (~~.~~0.051) and Arctic (~~.~~0.047) bacterial communities.

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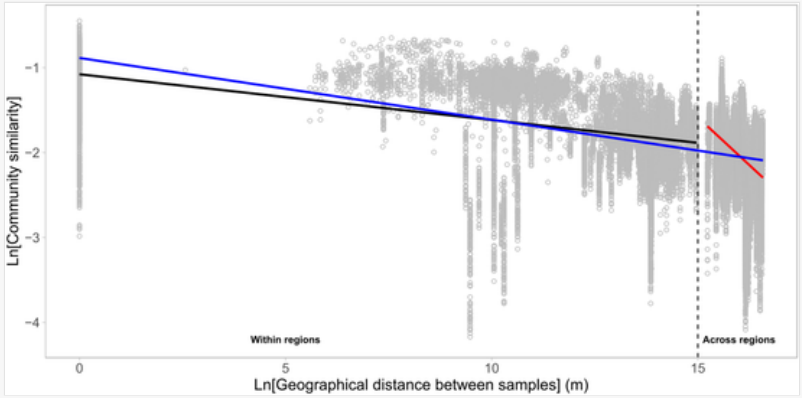
~~Fig. 6.~~Fig. 6



Correlation between community similarity (1 – Bray-Curtis distance) and geographic distance. The blue line denotes the least-squares linear regression across all spatial scales (regression line, y = –0.073x – 0.885; r = –0.481, $P < 0.001$). The black line denotes the linear regression of within regions (y = –0.054x – 1.077, r = –0.369, $P < 0.001$). The red line denotes the linear regression of across regions (y = –0.450x + 5.158, r = –0.387, $P < 0.001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The partial Mantel results revealed that both climatic distance (r = 0.259, P = 0.001) and geographic distance (r = 0.481, P = 0.001) were significantly correlated with the distribution of lake bacterial communities over all spatial scales (Table 2). Within the regions, the distribution of bacterial communities in the Arctic and Tibetan lakes was primarily governed by climatic variables. However, the geographic distance explained more variation of bacterial distribution in the Antarctic lakes (Table 2).

alt-text: Table 2

~~Table 2.~~ Table 2

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Mantel and partial Mantel tests for the correlation between bacterial community composition (Bray–Curtis distance) and climatic/geographic distances using Spearman's coefficient with 999 permutations.

	Factors	Controls	r	*P*
All	Clim		**0.259**	0.001
	Geo		**0.481**	0.001
	Clim	Geo	**0.097**	0.001
	Geo	Clim	**0.429**	0.001
Antarctica	Clim		**0.210**	0.002
	Geo		**0.252**	0.001
	Clim	Geo	–0.002	0.498
	Geo	Clim	**0.143**	0.001
Arctic	Clim		**0.512**	0.001
	Geo		**0.481**	0.001
	Clim	Geo	**0.412**	0.001
	Geo	Clim	**0.367**	0.001
TP	Clim		**0.460**	0.001
	Geo		**0.336**	0.001
	Clim	Geo	**0.372**	0.001
	Geo	Clim	**0.177**	0.001

All: the whole dataset, TP: Tibetan Plateau, Clim: climatic distance, measured as Euclidean distance using all the climatic variables, Geo: geographic distance. Data in bold present significant correlations ($P < 0.05$).

~~4.~~4 Discussion

~~4.1.~~4.1 Bacterial community composition across the three regions

The bacterial community composition varied significantly across lakes from the three different regions (Fig. 3 and Table S2). This finding supports the hypothesis that bacterial communities in the Tibetan lakes might be different to those of Arctic and Antarctic lakes. The observed dissimilarities in lake bacterial communities between the three regions might be due to an increased habitat heterogeneity over increasing geographic distance, which leads to geographic isolation and specific niches harboring different microbial communities (Langenheder and Lindstrom, 2019). Our results also showed differences in climatic factors between the three regions, such as MAT, MAP, NSR and evaporation which might structure the bacterial communities to a certain extent. The effect of climatic drivers (e.g., annual average temperature and annual solar radiation) has already been reported to explain the bacterial biogeography of terrestrial and lacustrine biofilms communities in the polar and temperate regions (Kleinteich et al., 2017). However, it is important to note that in the present dataset, all climatic factors were obtained according to the distinct geographic coordinates of the collected lake samples and were correlated with geographic coordinates (Dataset S1 and Fig. S2). This suggests that a part of the variation observed in community composition between the three regions may relate to geographic isolation of lake planktonic bacteria.

Resolving spatial patterns of diversity have long been one of the core objectives in ecological research. Climatic factors, e.g., temperature and precipitation, are regarded as the important drivers underlying diversity gradients over broad spatial scales (Gaston, 2000; Wang et al., 2016). For instance, our study indicated that the MAT, MAP and evaporation were of importance in determining the spatial pattern of bacterial OTU richness in the Arctic lakes (Table 1). It is possible that within the Arctic, higher MAT, MAP and evaporation may lead to higher runoff transport more terrestrial microbes into the lakes, which can affect the spatial variability in bacterial taxonomic richness in these lakes (Burpee et al., 2016; Comte et al., 2018; Crump et al., 2012). Indeed, studies of the bacterial communities in boreal freshwater networks have shown that hydrologic connectivity plays an important role in the transport of microbes into lakes from the surrounding catchment (Comte et al., 2017; Niño-García et al., 2016; Ruiz-González et al., 2015; Wisnoski et al., 2020). In addition, it should be noted that some unmeasured local environmental factors, such as the water residence time (Crump et al., 2012; Lindström et al., 2005; Ruiz-González et al., 2015; Niño-García et al., 2016), pH (Crevecoeur et al., 2019; Niño-García et al., 2016), salinity (Logares et al., 2013; Yang et al., 2019) and total phosphorus or total organic carbon (Comte et al., 2017), may be important as well in affecting lake bacterial richness and may explain to a certain extent the observed spatial patterns in communities within and across the three regions.

~~4.2.~~4.2 Spatial distribution of shared and endemic OTUs

Geographic isolation probably lead to the percentage of shared bacterial OTUs between the three regions lower than those unique OTUs occurring in each region (Fig. 4 and Fig. S4). However, the few shared OTUs did account for a considerable proportion of the total sequences (36.4%), further substantiating the idea that the most ubiquitous taxa are often the most abundant (Niño-García et al., 2016; Salazar et al., 2016). In general, distant and isolated habitats are expected to have specific communities harboring large proportions of endemic taxa, while geographically closer habitats have large proportions of shared species with more cosmopolitan species (Hahn et al., 2015; Martiny et al., 2006). Consistent with this assumption, the proportion of shared bacterial OTUs between each two regions (10.9% between Arctic and the Tibetan Plateau, 6.8% between Antarctica and the Tibetan Plateau and 2.5% between Arctic and Antarctica, respectively) decreased with increasing geographic distance. Such patterns may partly be explained by the fact that taxa vary in dispersal ability, making dispersal probability not entirely random among species (Hanson et al., 2012). Generally, species with high dispersal ability will weaken the role of dispersal limitation and lead to little variation in the community composition, whereas species with lower dispersal ability could increase community variation (Zhou and Ning, 2017).

Taxonomic analyses revealed that species affiliated with Burkholderiales (Betaproteobacteria), Actinomycetales (Actinobacteria), as well as members of the phylum Bacteroidetes dominated the shared OTUs (Fig. 5a and Fig. S3a). These bacteria are well known for their organic matter degradation abilities and cosmopolitan distribution in polar and non-polar lakes (Crump et al., 2012; Daniel et al., 2016; Logares et al., 2013; Newton et al., 2011). For example, members of Actinomycetales are found in many habitats ranging from terrigenous, marine and aquatic environments, especially can survive in aerial environments (Goodfellow and Williams, 1983; Subramani and Aalbersberg, 2012). It is reported that the cosmopolitan distribution of Actinomycetales may be partly due to their ability to form spores, which can travel long distances in the air (Hervas et al., 2009; Weber and Werth, 2015). The most abundant endemic taxa identified in each of region (e.g., Bacteroidetes in Antarctica, Betaproteobacteria in Arctic and Actinobacteria in the Tibetan lakes) were similar to those dominant shared taxa across the three regions. This agrees with previous studies on large-scale distributions of free-living freshwater and marine bacterial communities, showing that some taxa display very different spatial distributions and environmental preferences and tolerances (Niño-García et al., 2016; Ruiz-González et al., 2019). Additionally, certain bacterial lineages were apparently confined uniquely to specific regions. For example, endemic taxa assigned to the orders of Chthoniobacterales and Puniceicoccales within the phylum Verrucomicrobia and the order Pirellulales within the phylum Planctomycetes were detected as abundant in the Tibetan lakes but not in any of the other two regions (Fig. S3d). Members of Verrucomicrobia and Planctomycetes are known for their abilities to degrade phytoplankton-derived carbohydrates, and have been found abundant in nutrient-poor aquatic environments (Newton et al., 2011). A previous study on phytoplankton assemblages in 38 Tibetan lakes

reported that some planktonic phototrophic algae, such as Bacillariophyceae, Chlorophyceae and Cyanobacteria were the dominant primary producers in the Tibetan lakes (Yang et al., 2017). So the prevalence of Verrucomicrobia and Planctomycetes taxa in the Tibetan lakes may partly due to their abilities to cope with low nutrient availability through an access to phytoplankton-derived organic carbon.

~~4.3.~~4.3 Scale-dependent distance-decay patterns

The relationships between bacterial community similarity and geographic distance varied over regional to continental scales (Fig. 6 and Fig. S5). Spatial patterns of microbial community provide insights into the underlying ecological mechanisms (Langenheder and Lindstrom, 2019; Martiny et al., 2011). Over all spatial scales, both the climate and spatial variables appeared to be important in shaping the distance-decay pattern of bacterial community (Table 2). This is consistent with studies of ammonia-oxidizing bacterial communities in marshes at different scales (within marshes, among marshes and among continents) showing that the contribution of local environmental and spatial factors in shaping microbial distribution patterns are likely scale dependent (Martiny et al., 2011). Within the Arctic and Tibetan Plateau regions, lake bacterial communities were more strongly correlated with climatic variables than with geographic distance (as a proxy for dispersal limitation). However, the effect of dispersal limitation became stronger across regions (Partial Mantel $r = 0.429$, $P = 0.001$ versus $r = 0.097$, $P = 0.001$; Table 2). Our findings furthermore corroborate the ideas of Langenheder and Lindstrom (2019), who declare that at larger spatial scales, species sorting is primarily driven by climatic drivers (e.g., precipitation and temperature), but dispersal limitation is expected to become increasingly important.

Our results showed that geographic distance negatively correlated with the similarity of bacterial communities, while climatic variables did not correlate with bacterial communities after controlling for geographic distance in the Antarctic lakes (Partial Mantel $P = 0.498$; Table 2). It can thus be suggested that climatic variables in the Antarctica are spatially auto-correlated. Regarding the relationships between distance and community dissimilarity among the three regions, Antarctica showed the highest differences across lake communities and appeared much more dispersed than the other two regions. (Fig. 3). This is likely because the Antarctic lakes were located extremely far away (Fig. 1), which probably led to a certain degree of geographic isolation but also to large differences in the environmental variables or biotic factors between the Antarctic lakes.

~~5.~~5 Conclusions

In summary, this study provides a comparison of the composition and biogeography of bacterial communities in the lakes of the Tibetan Plateau, Antarctica and the Arctic. Our results revealed significant differences in bacterial OTU richness and community composition across the three regions. The severe climate of these regions as well as their geographic isolation has led to a high level of endemism. Environmental parameters related to climatic conditions and geographic distance had strong effects on bacterial community similarity at both regional and continental scales, implying that climate-driven species sorting and spatial effects were important in controlling the spatial distributions of lake bacterial community composition in the lakes of the Tibetan Plateau, Antarctica and the Arctic. A limitation of this study is the paucity of local environmental factors (e.g., pH, salinity and water residence time), which are is likely to affect the biogeography of lake bacterial communities across the three regions. Notwithstanding this limitation, this study offers valuable insight into how the degree of physical isolation explains differences in lake bacterial communities between the Tibetan Plateau and the polar regions.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.142248.

Uncited references

~~Jungblut et al., 2016~~

~~Yao et al., 2012~~

CRediT authorship contribution statement

Keshao Liu: Conceptualization, Investigation, Methodology, Data curation, Writing - original draft. **Tandong Yao:** Conceptualization. **David A. Pearce:** Writing - review & editing. **Nianzhi Jiao:** Conceptualization. **Yonghui Zeng:** Writing - review & editing. **Bixi Guo:** Methodology, Visualization. **Yongqin Liu:** Conceptualization, Supervision, Funding acquisition.

Declaration of competing interest~~s~~

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The corrections made in this section will be reviewed and approved by a journal production editor. The newly added/removed references and its citations will be reordered and rearranged by the production team.

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Graphical abstract

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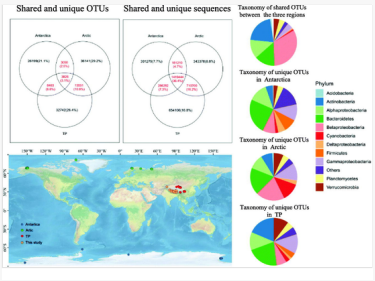

Highlights

- The bacterial diversity and community composition varied significantly across lakes from the Tibetan Plateau, Antarctica and the Arctic.
- Geographic distance limits the exchange of species between the Tibetan Plateau, Antarctic and the Arctic lakes and lead to a high proportion of endemic species within each region.
- The few shared taxa were often the most abundant.
- Both climatic conditions and geographic distance had an effect on the spatial distribution of bacterial communities at both regional and continental scales.

A total of 84 water samples used in this study were collected from 19 Tibetan lakes during several field campaigns in 2016 and 2017 summers, respectively (*Dataset S1*). The selected lakes covered an area from 75.05°E to 90.86°E longitudinally and 28.89°N to 38.46°N latitudinally. A minimum of triplicate surface water samples (~0.5 m depth) from the epilimnion were collected with a Schindler sampler at the center of each lake. For microbial DNA collection, 1 L water samples were filtered through a 20 μm mesh (Millipore, USA) to remove large particles or organisms, and subsequently through a 0.22 μm pore size polycarbonate filters (47 mm diameter; Millipore). The filters were immersed in 1.8 mL of RNAlater (Life Technologies, USA) to stabilize nucleic acids and then stored at −80 °C in the laboratory until further processing. Environmental DNA from each sample was extracted using the MP FastDNA~~TM~~™ SPIN DNA kit (MP Biomedicals, CA, United States) and amplified using primers targeting the V4 region of the 16S rRNA gene (515F (5′-GTGCCAGCMGCCGCGGTAA-3′, and 806R 5′-GGACTACHVGGGTWTCTAAT-3′) of the Earth Microbiome Project (EMP) (Caporaso et al., 2010). The amplicons were mixed in equimolar concentrations and sequenced on an Illumina MiSeq Sequencer (Illumina, San Diego, CA) with a paired-end strategy (2 × 250 bp). Web of Science (2010–2019) was searched for literature and the NCBI Sequence Read Archive database for deposited sequences using the combined keywords (‘Antarctic’ or ‘Arctic’ or ‘Tibetan’) and (‘lake microbial’ or ‘lake bacterial’). Since cloning-based and high-throughput sequencing methods are not directly comparable, only studies or data generated from high-throughput sequencing methods were included. Based on the criteria described above, a total of 147 water samples were collected from 32 Tibetan lakes, 81 water samples from 9 Antarctic lakes and 154 water samples from 16 Arctic lakes (Fig. 1; *Dataset S1*). Due to poor accessibility and harsh field conditions, there were fewer studies or available datasets on Antarctic lakes than those in the Arctic and Tibetan Plateau. It should note that in the current study only planktonic communities were considered so that the three datasets are directly comparable.

The bacterial community composition varied significantly across lakes from the three different regions (Fig. 3 and Table S2). This finding supports the hypothesis that bacterial communities in the Tibetan lakes might be different to those of Arctic and Antarctic lakes. The observed dissimilarities in lake bacterial communities between the three regions might be due to an increased habitat heterogeneity over increasing geographic distance, which leads to geographic isolation and specific niches harboring different microbial communities (Langenheder and Lindstrom, 2019). Our results also showed differences in climatic factors between the three regions, such as MAT, MAP, NSR and evaporation which might structure the bacterial communities to a certain extent. The effect of climatic drivers (e.g., annual average temperature and annual solar radiation) has already been reported to explain the bacterial biogeography of terrestrial and lacustrine biofilms communities in the polar and temperate regions (Kleinteich et al., 2017). However, it is important to note that in the present dataset, all climatic factors were obtained according to the distinct geographic coordinates of the collected lake samples and were correlated with geographic coordinates (*Dataset S1* and Fig. S2). This suggests that a part of the variation observed in community composition between the three regions may relate to geographic isolation of lake planktonic bacteria.

The following ~~is~~ are the supplementary data related to this article.

Multimedia Component 1

Dataset S1

The basic information of lake samples used in this study.

alt-text: Dataset S1

Multimedia Component 2

Supplementary material

alt-text: Image 1

Queries and Answers

Q1

Query: Please provide a definition for the significance of bold in the table.

Answer: Data in bold present significant correlations ($P < 0.05$).

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Q8

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Q9

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Q12

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